

New fluorescent antibody test for the serological diagnosis of syphilis

FTA blocking technique using an anti-Reiter serum^{*}

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The FTA-ABS test (Hunter, Deacon, and Meyer, 1964) has a high sensitivity due to the low dilution (1 : 5) of the test serum. The specificity of this test is also of a high order, since the principle on which it is based ensures removal of the group treponemal protein antibodies.

In order to have a test just as sensitive and specific as the FTA-ABS test, a technique has been developed based on a different principle and mechanism, namely a single-stage method for blocking the reaction of the *Treponema pallidum* (Tp) non-specific antibodies.

The method is similar in principle to the fluorescent antibody test used by Lind (1967, 1968) to identify the gonococcus, and consists in making the group protein antibody in the serum of the subject under test and the group protein antibody obtained experimentally from rabbits injected with Reiter treponeme compete for the group antigen carried by Tp.

Király and Kováts (1967) used a similar technique but it is performed in two successive steps. First, the *Treponema pallidum* smear is incubated with an anti-Reiter treponeme serum, and then the test serum diluted 1 : 5 is placed on the antigen and left in contact with it.

Material and methods

Before the FTA blocking technique using rabbit anti-Reiter serum can be used in ordinary practice it must be shown to have a sensitivity and specificity of an adequate standard.

The value of the technique was tested by comparing the results given by this test and by the FTA-ABS test on 174 sera.

The distribution of test sera was as follows:

- (a) 14 from subjects who definitely were not syphilitic;
- (b) 53 from subjects giving a biological false positive FTA 5 test;
- (c) 10 from syphilis cases with sera giving a negative FTA 200 and positive FTA 5 and FTA-ABS;
- (d) 40 from syphilis cases with sera giving a FTA titre from 200 to 800;
- (e) 57 from syphilis cases with sera giving a high FTA titre (greater than 800).

FTA blocking technique using a rabbit anti-Reiter treponeme serum

The antigen used as indicator in this test, as in the FTA-ABS test, was a smear of *Treponema pallidum*. The test serum was diluted 1 : 5 in a rabbit anti-Reiter serum prepared in the laboratory. This dilution was then placed on the fixed antigen and left in contact with it for 30 min. at 37°C.

A first washing with phosphate buffered saline pH 7.2 was followed by 30 min. contact at room temperature with rabbit anti-human globulin conjugate*. After further washing with PBS 7.2, the preparation was dried, mounted between slide and cover slip, and examined under a fluorescence microscope (Thivolet, Salussola, Sepetjian, and Monier, 1969).

The anti-Reiter treponeme sera were prepared from rabbits which had given a negative FTA-ABS test before beginning immunization. The following procedure was used for all immunizations.

The inoculum was prepared from a culture of Reiter treponemes grown for 8 days in spirochate broth (BBL) to which 10 per cent. of sterile horse serum had been added.

10 ml. of this culture were centrifuged at 1,800 rev./min. for 5 min. to remove large particles. Further centrifugation for 20 min. at 6,000 rev./min. gave a sediment of treponemes which was re-suspended in 2 ml. of sterile normal saline to give the inoculum used.

This antigen was introduced into rabbits first by intravenous then by subcutaneous injection. A first series of three intravenous injections were given at 5-day intervals, using 1 ml., 1 ml., and 2 ml. volumes of the Reiter treponeme suspension.

The second series of injections was administered subcutaneously at 7-day intervals, using 1 ml., 1 ml., and 2 ml. volumes of suspension, respectively. For the first two subcutaneous injections the inoculum was mixed with 1 ml. of Freund's complete adjuvant.

The rabbit sera obtained in this manner were titrated by a quantitative FTA test before being used in the FTA blocking test, and initially only those sera with an FTA titre equal to or greater than 12,800 were retained for the latter test. Under these conditions three batches of sera from three different animals, rabbits Nos. 46, 53, and 1, were used.

A comparative study was made of the action of each of these three sera in the FTA blocking test.

Tests on human sera

The following tests were also carried out on all sera of human origin subjected to the FTA blocking test: the FTA 5 test (1 : 5 dilution of serum in PBS), the FTA 200 test (quantitative wherever necessary) and finally the FTA-ABS test (serum diluted 1 : 5 in a Reiter treponeme suspension).

Results

The results given by the FTA blocking, FTA 5, FTA 200, and FTA-ABS tests for the 174 sera studied are shown in Tables I and II.

In the great majority of cases (171 sera out of 174) the results of the FTA blocking and FTA-ABS tests agree (Table I). Disagreement, however, was observed in three cases (Table II).

To prove that normal rabbit serum giving a negative response to the FTA 200 test was incapable of producing a blocking effect similar to that caused by our anti-Reiter serum, we used the sera from fifteen healthy rabbits as the diluent when five human sera giving a false positive FTA 200 test were subjected to the FTA blocking test. In no case was the original positive finding reversed.

Discussion

The choice of the one-step blocking test was realized after a comparative study of a two-step technique similar to that described by Király and Kováts (1967). The latter did not regularly produce the blocking effect wanted.

TABLE I 171 cases in which the FTA-ABS test and the FTA blocking test gave the same result when carried out on a series of 174 sera

FTA 200 test	Category of patient		No. of patients in each category	Test serum diluted 1:5 in		
				PBS (FTA 5)	Sorbing agent FTA-ABS	Anti-Reiter serum Nos 46 or 53 (FTA blocking)
Negative	Without syphilis	FTA 5 = 0	14	neg.	neg.	neg.
		FTA 5 false pos.	45	pos.	neg.	neg.
	With syphilis	FTA 5 = +	10	pos.	pos.	pos.
Positive	Without syphilis	FTA 200 false pos.	5	pos.	neg.	neg.
	With syphilis	$800 \geq T \geq 200$	40	pos.	pos.	pos.
		$>T800$	57	pos.	pos.	pos.

T = titre

TABLE II Three cases in which the FTA-ABS test and the FTA blocking test gave a different result when carried out on a series of 174 sera

Serum no.	FTA	Test serum diluted 1 : 5 in		
		PBS (FTA 5)	Sorbing agent (FTA-ABS)	Rabbit anti-Reiter serum No. 53 (FTA blocking)
1	negative	positive	negative	positive
2	negative	positive	negative	positive
3	T = 400	positive	negative	positive

T = titre

Table I shows the one-step blocking test to have a high degree of sensitivity and specificity.

In ten cases in which the lack of sensitivity of the FTA 200 test led to a negative result, both the FTA-ABS and the FTA blocking test were positive. Whenever the FTA test was positive and an actual case of syphilis was involved, regardless of whether the titre was low (less than or equal to 800 : 40 sera) or high (over 800 : 57 sera), the blocking test was consistently positive.

The specificity of the blocking test would seem to be high, since in the five cases in which the FTA 200 test gave a biological false positive reaction and in the 45 cases in which the FTA 5 test gave a false positive result, the blocking test was negative as also was the FTA-ABS test. In addition, all fourteen subjects without syphilis were negative in the blocking test.

We found, however, that in three cases the results of the FTA-ABS and FTA blocking tests differed (Table II).

In these cases the inhibiting power of the anti-Reiter serum used was apparently inadequate, since the FTA-ABS test and TPI test were negative and the FTA blocking test positive. This raises the problem of anti-Reiter sera varying in activity and efficacy from one production batch to another.

The human sera involved, Nos. 1, 2, and 3 (Table II), had been treated by rabbit anti-Reiter serum No. 53, which subsequently appeared to exert an inadequate blocking effect in certain cases in spite of its FTA titre of 12,800. The test sera in this preliminary study were therefore treated either with one of the two anti-Reiter sera, No. 46 and No. 53, alone, or in 23 cases with both these blocking sera together.

The results of the FTA blocking test were observed to disagree with the FTA-ABS results only in the three cases in which serum No. 53 had been used.

This shows that the preparation of anti-Reiter serum with an adequate blocking effect is one of the difficulties of the recommended technique.

Although the FTA titre of these experimentally prepared sera may be helpful when selecting a blocking serum, other factors, in particular the reactivity of the anti-Reiter antibody, are also likely to influence the final result.

When anti-Reiter serum obtained after immunization has an FTA titre under 12,800 and shows an inadequate blocking effect, it may be concentrated by freeze-drying to give acceptable blocking in the FTA blocking test without any risk of giving negative results on sera from syphilis patients with low positive FTA titres (200 or 400). This was demonstrated for several preparations of anti-Reiter serum.

The preparation of anti-Reiter sera thus seems to need more thorough investigation aimed at:

- (1) Modifying the immunization procedure;
- (2) Attempting to find an animal species other than the rabbit with superior immunological reactivity to the Reiter treponeme;
- (3) Producing serum containing antibody with greater reactivity;
- (4) Using different methods to try to increase the antibody concentration of the different experimentally produced sera;
- (5) Using only the fraction containing immunoglobulin as the blocking factor.

Summary

A new fluorescent antibody test is described which has the same purpose as the FTA-ABS test but uses a different principle. The new test, known as the FTA blocking test, is simple to carry out and uses a rabbit anti-Reiter serum instead of the sorbing agent. The results obtained on 174 sera of human origin show the sensitivity and specificity of this new test to be practically the same as those of the FTA-ABS test.

References

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Une nouvelle réaction d'immunofluorescence pour le diagnostic sérologique de la syphilis

FTA-inhibition utilisant un sérum anti-Reiter

SOMMAIRE

Une nouvelle réaction d'immunofluorescence ayant les mêmes objectifs que le FTA-ABS mais reposant sur un principe différent, est proposée par les auteurs. Ce nouveau test, de réalisation simple, dénommé FTA-inhibition utilise à la place du 'Sorbet' un sérum de lapin anti-Reiter. Les résultats obtenus sur 174 sérums d'origine humaine montrent que les critères de sensibilité et de spécificité de cette nouvelle réaction sont pratiquement superposables à ceux du FTA-ABS.